

UK-1, A NOVEL CYTOTOXIC METABOLITE FROM *Streptomyces* sp. 517-02

II. STRUCTURAL ELUCIDATION

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The structure of UK-1 isolated from the mycelium of *Streptomyces* sp. 517-02 was elucidated to be a novel benzoxazole dimer derivative (1) on the basis of spectroscopic methods.

A novel metabolite with potent cytotoxic activity against B16, HeLa and P388 cells, UK-1, was isolated from the mycelium of *Streptomyces* sp. 517-02 as described in a previous paper¹⁾. The structure of UK-1 was elucidated to be a dimeric benzoxazole derivative constituted of two moles of 3-hydroxyanthranilic acid and one mole of salicylic acid on the basis of some spectroscopic methods (Fig. 1). The structure determination studies of UK-1 are described in this paper.

Results and Discussion

The IR spectrum of UK-1 (1) described in a previous paper showed a strong absorption based on ester group at 1725 cm^{-1} and the UV spectrum of 1 suggested the existence of conjugated system in the molecule. The molecular formula of 1 was determined as $\text{C}_{22}\text{H}_{14}\text{N}_2\text{O}_5$ from the HREI-MS (M^+ : m/z 386.0913, Calcd for $\text{C}_{22}\text{H}_{14}\text{N}_2\text{O}_5$, 386.0903) as the base peak and ^{13}C NMR spectral data. Other fragment ions were observed at m/z 354.0606 (Calcd for $\text{C}_{22}\text{H}_{14}\text{N}_2\text{O}_5 \cdot \text{CH}_3\text{OH}$, 354.0572) and m/z 328.0888 (Calcd for $\text{C}_{22}\text{H}_{14}\text{N}_2\text{O}_5 \cdot \text{HCOOCH}_3$, 328.0928) in the HREI-MS spectrum of 1. The absorption of a hydroxyl group could not be observed in the IR spectrum of 1 but the signal based on a strong hydrogen bonded hydroxyl group appeared at δ 11.9 ppm in the ^1H NMR spectrum in CDCl_3 . Moreover, 1 afforded its mono-methyl ether, Me-UK-1 (2), by methylation with methyl iodide and anhydrous potassium carbonate in dry acetone. The IR spectrum of 2 showed the absorption based on an ester group at ν_{max} 1710 cm^{-1} . In the ^1H and ^{13}C NMR spectra of 2, the signals of a methoxyl group appeared at δ 4.09 and δ 56.22, respectively showing to be the monomethyl derivative of 1. Alkaline hydrolysis of 1 furnished the corresponding carboxylic acid, DeMe-UK-1 (3). The absorption based on a carboxyl group appeared at ν_{max} $2500\sim 3100$ and 1690 cm^{-1} in the IR spectrum of 3 and the signal of a methoxycarbonyl group disappeared in the ^1H NMR.

The ^1H and ^{13}C NMR spectral data of 1, 2 and 3 are shown in Table 1. The assignments of proton and carbon signals were done using ^1H - ^1H COSY and ^1H - ^{13}C COSY spectra (Fig. 2) and

Fig. 1. Structures of UK-1 (1) and its derivatives.

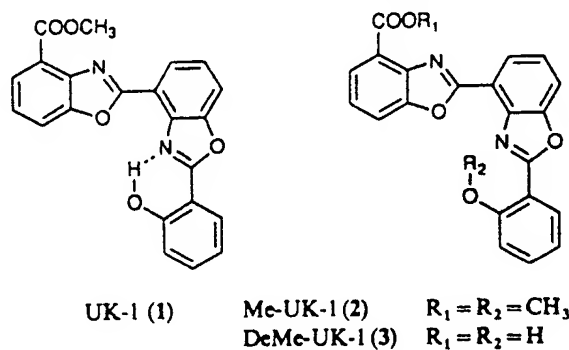


Table 1. NMR spectral data for benzoxazoles.

No.	UK-1 (1) ^a		Me-UK-1 (2) ^a		DeMe-UK-1 (3) ^b		2-o-Methoxybenzoxazole (5) ^a		2-o-Hydroxybenzoxazole (6) ^a	
	¹³ C shift	¹ H shift	¹³ C shift	¹ H shift	¹³ C shift	¹ H shift	¹³ C shift	¹ H shift	¹³ C shift	¹ H shift
1	151.41		151.86		151.83					
2	141.59		141.64		142.05					
3	122.67		122.39		122.98					
4	127.41	8.07 dd	127.11	8.06 dd	127.82	8.39 d				
5	124.83	7.44 t	124.47	7.44 t	125.32	7.47 t				
6	114.96	7.82 dd	115.34	7.91 dd	114.71	7.85 d				
7	161.81		163.20		159.93 ^c					
8	117.77		118.45		118.00		120.25	7.59 d	119.55	7.69 d
9	138.74		140.75		138.78		142.24		140.14	
10	149.98		151.47		150.28		150.53		149.23	
11	113.69	7.72 dd	113.88	7.78 dd	114.20	7.79 d	110.48	7.81 d	110.64	7.71 d
12	125.22	7.48 t	124.76	7.50 t	125.69	7.47 t	124.28	7.35 t	125.01	7.36 t
13	125.33	8.31 dd	125.79	8.43 dd	125.88	8.42 d	124.90	7.32 t	125.37	7.33 t
14	164.62		163.89		164.57		161.87		162.98	
15	110.06		116.00		110.57		116.50		110.64	
16	159.68		159.03		159.93 ^c		158.62		158.85	
17	117.84	7.15 d	112.34	7.10 d	118.16	7.31 d	112.26	7.08 d	117.47	7.12 d
18	134.24	7.46 t	133.37	7.54 t	134.71	7.51 t	132.73	7.49 t	133.56	7.42 t
19	119.56	7.00 td	120.83	7.13 t	120.04	7.05 t	120.77	7.07 t	119.27	6.98 t
20	127.21	8.01 dd	131.95	8.32 d	127.65	8.04 d	131.40	8.13 d	127.15	8.01 d
C-O	166.12		165.86		167.81					
OMe	52.53	4.17 s	52.32	4.10 s			56.26	4.01 s		
OH		11.90 s	56.22	4.09 s						11.42 s

^a In CDCl₃.^b In pyridine-*d*₅.^c Overlapped signal.

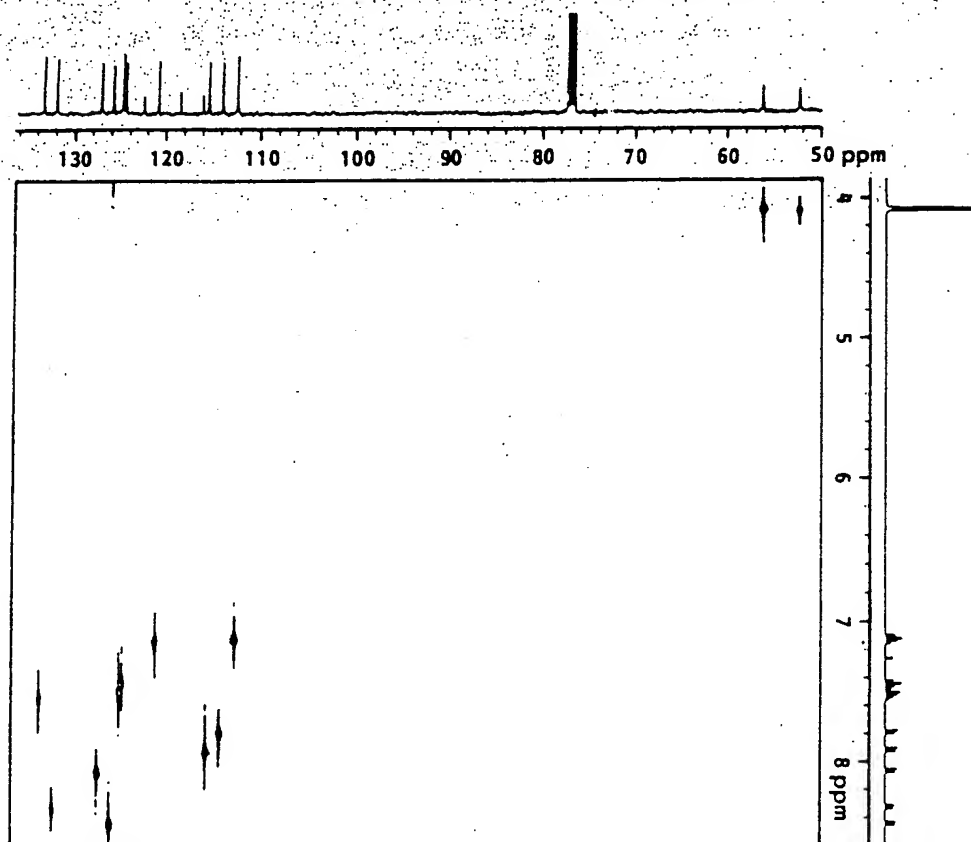
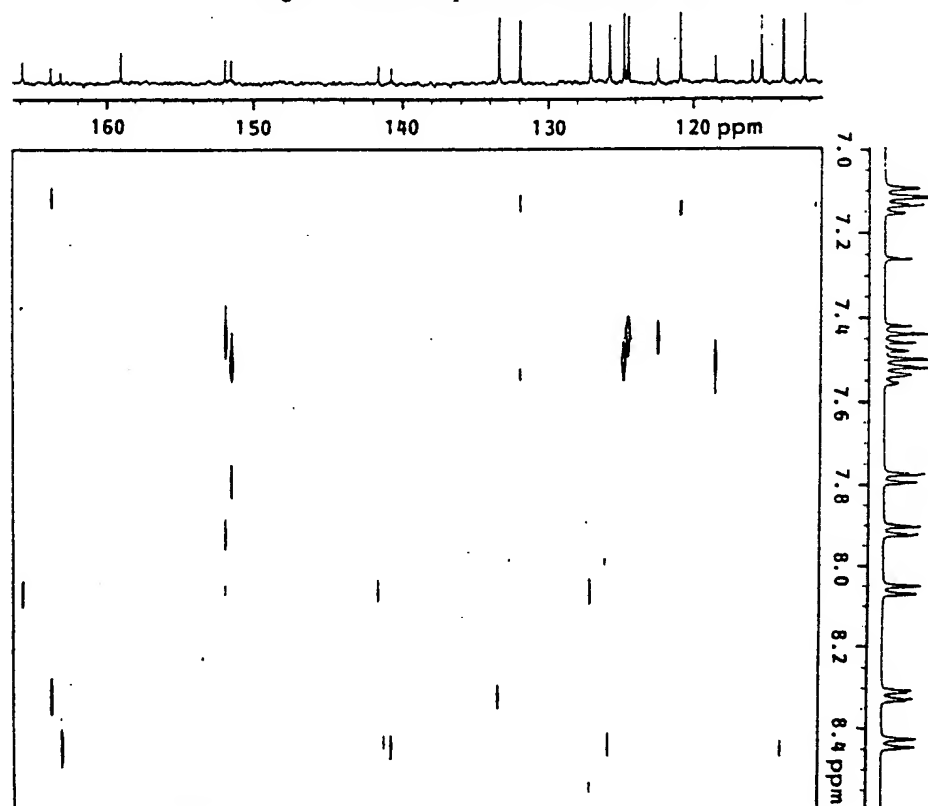
Fig. 2. ^1H - ^{13}C COSY spectrum of Me-UK-1 (2).

Fig. 3. COLOC spectrum of Me-UK-1 (2).

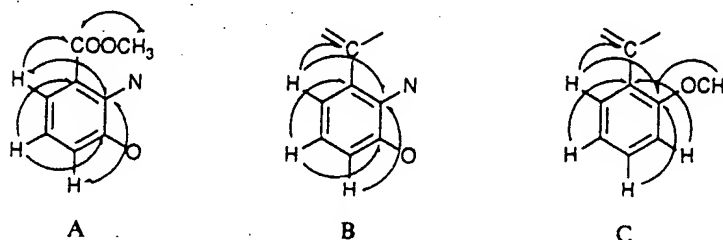


correlation spectroscopy *via* long-range couplings (COLOC) measurement (Fig. 3). These results revealed the partial structure A, B and C in 2 as shown in Fig. 4, and some possible formulae as the structure of 1 were estimated by the combination of these partial structures.

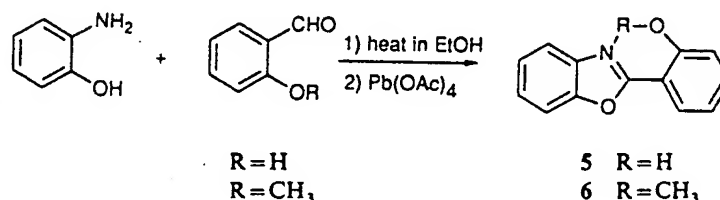
The ^{13}C NMR parameters in the benzene ring of 1 are consistent with the values calculated on the basis of the chemical shift of carbons in the benzene ring of benzoxazole (4)². Moreover, the benzoxazole derivatives (5 and 6) were prepared from *o*-aminophenol and *o*-anisaldehyde or salicylaldehyde, respectively (Scheme 1)³, and the ^{13}C chemical shifts of these derivatives were in good accordance with those of 1 and 2 (Table 1).

From these results, the structure of UK-1 was deduced to be formula 1, the novel benzoxazole dimer derivative.

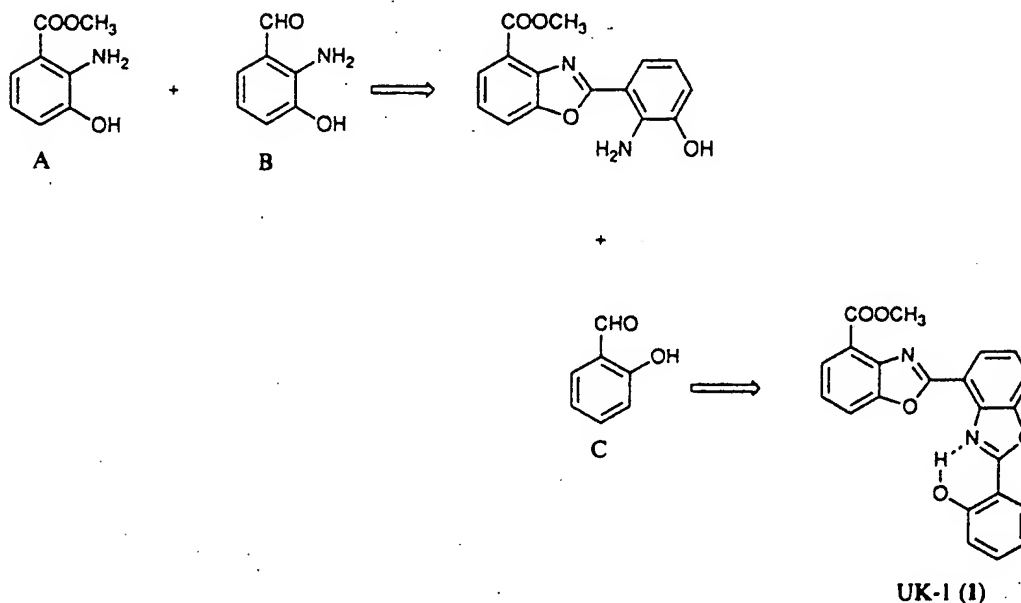
Fig. 4. Partial structures of Me-UK-1 (2) and the correlation of ^1H - ^{13}C long-range couplings.



Scheme 1.



Scheme 2. Estimation of biosynthesis for UK-1.



Both of the fragments A and B of 1 are related to 3-hydroxyanthranilic acid, one of the catabolic products *via* kynurenine and the fragment C is a reduced product of salicylic acid. The benzoxazoles 5 and 6 were easily prepared by oxidation of SCHIFF's bases derived from *o*-aminophenol and corresponding aldehydes. It seems that UK-1 was biosynthesized by oxidation of the SCHIFF's base prepared from methyl 3-hydroxyanthranilate and 3-hydroxyanthranilaldehyde produced in the decomposition pathway of L-tryptophan, followed to the preparation of SCHIFF's base with salicylaldehyde and the oxidative ring closure reaction of SCHIFF's base (Scheme 2). Studies on the biosynthesis of 1 are now in progress.

Experimental

MS and NMR

EI-MS and HREI-EI-MS spectra were obtained with a JEOL-JMS-AX 500 mass spectrometer. All NMR spectra were recorded on a JEOL-JNM-GX-400 spectrometer operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. Tetramethylsilane was used as an internal reference for ^1H NMR in the CDCl_3 solution. For the ^{13}C chemical shift reference, the ^{13}C peak at δ 77.03 ppm of CDCl_3 was used. The ^1H peak at δ 7.0 ppm and ^{13}C peak at δ 122.4 ppm of pyridine- d_5 were used as the internal references for NMR measurement in pyridine- d_5 .

Methylation of 1

Anhydrous potassium carbonate (2 g) was suspended in a solution of 50 mg of 1 and 0.5 ml of methyl iodide in 20 ml of dry acetone, and the mixture was refluxed for 5 hours. After filtration, acetone was evaporated and the residual mixture was dissolved in CH_2Cl_2 , followed to chromatographical purification on a silica gel column. By recrystallization with methanol, 2 was obtained as colorless needles in quantitative yield. 2; MP 145~147°C, IR (nujol) 1710, 1600, 1590, 1580, 1540 cm^{-1} , HREI-MS (M^+) = m/z 400.1067 (Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_5$, 400.1075), NMR, see Table 1.

Alkaline Hydrolysis of 1

Alkaline hydrolysis of 1 with aqueous NaOH in a pyridine solution at room temperature afforded 3. 3; MP > 300°C, IR (nujol) 2500~3100, 1690, 1600, 1560 cm^{-1} , NMR, see Table 1.

Preparation of 2-Phenylbenzoxazoles

2-Phenylbenzoxazoles were prepared according to the procedure of STEPHENS and BOWER³⁾. Namely, *o*-aminophenol dissolved in ethanol was mixed with the corresponding aldehyde, boiled for 10 minutes and cooled. The product obtained by filtration and recrystallization from ethanol gave a red SCHIFF's base. The treatment of the SCHIFF's base with lead tetraacetate in glacial acetic acid afforded the 2-phenylbenzoxazole, 4 or 5.

4; The yield of SCHIFF's base from *o*-aminophenol (1.1 g) and *o*-anisaldehyde (1.4 g) was 1.04 g (70%). The dehydrogenation of SCHIFF's base (140 mg) with lead tetraacetate (211 mg) in acetic acid (3.5 ml) afforded 2-*o*-methoxyphenylbenzoxazole (38 mg). ^1H and ^{13}C NMR, see Table 1.

5; The yield of SCHIFF's base from *o*-aminophenol (1.1 g) and salicylaldehyde (1.2 g) was 2.24 g (100%). The dehydrogenation of SCHIFF's base (1.23 g) with lead tetraacetate (2.1 g) in acetic acid (20 ml) afforded 2-*o*-hydroxyphenylbenzoxazole. ^1H and ^{13}C NMR, see Table 1.

Acknowledgments

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References

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